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REVIEW

Valorization of Proteins from Co- and By-Products from the Fish and Meat Industry

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Abstract Large volumes of protein-rich residual raw materials, such as heads, bones, carcasses, blood, skin, viscera, hooves and feathers, are created as a result of processing of animals from fisheries, aquaculture, livestock and poultry sectors. These residuals contain proteins and other essential nutrients with potentially bioactive properties, eligible for recycling and upgrading for higher-value products, e.g. for human, pet food and feed purposes. Here, we aim to cover all the important aspects of achieving optimal utilization of proteins in such residual raw materials, identifying those eligible for human consumption as co-products and for feed applications as by-products. Strict legislation regulates the utilization of various animal-based co- and by-products, representing a major hurdle if not addressed properly. Thorough understanding and optimization of all parts of the production chain, including conservation and processing, are important prerequisites for successful upgrading and industrial implementation of such products. This review includes industrially applied technologies such as freezing/cooling, acid preservation, salting, rendering and protein hydrolysis. In this regard, it is important to achieve stable production and quality through all the steps in the manufacturing chain, preferably supported by at- or online quality control points in the actual processing step. If aiming for the human market, knowledge of consumer trends and

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awareness are important for production and successful introduction of new products and ingredients.

Keywords Food and feed applications · Enzymatic hydrolysis · Downstream processing · Bioactivity · Analytical chemistry · Consumers

Abbreviations

ABPs	Animal by-products
BAPs	Bioactive peptides
BSE	Bovine spongiform encephalopathy
EU	European Union
FTIR	Fourier-transform infrared
TSE	Transmissible spongiform encephalopathy

1 Introduction

The rapid growth, urbanization and increasing prosperity of the world population demand improved utilization of existing protein sources along with development of new and sustainable food production. The global demand for protein is expected to double by the year 2050. This is due to not only population growth but also increased recognition of the important role of protein in a healthy diet in general and especially for children and the growing elderly population [1]. Fish and meat products are important protein sources in the human diet and contain essential amino acids, minerals and vitamins. In industrial fish and meat processing, the main goal is to process the main products, such as fillets, chops and mince. However, these processes generate huge amounts of protein-rich residual raw materials. About 40–60 % of the total weight of animals and fishes are classified as residuals. This includes heads, bones, carcasses, blood, skin, viscera, hooves and feathers, depending on the species. Much of this material has great potential for higher-value applications in food and feed. This review focusses on how to increase the value of such material derived from livestock, poultry, fisheries, and aquaculture sectors. Such residuals can be divided into co-products, which can be used for human consumption, and by-products, which cannot (Fig. 1). Applications of by-products are strictly regulated; this review provides an overview of the most important European regulations. Moreover, it presents more established industrial processes, e.g. ensilage and rendering, as well as emerging processes, i.e. enzymatic hydrolysis, for processing of fish and meat co- and by-products. When selecting the most suitable process for each specific situation, a trade-off is possible. Established processes, with relatively easy production and low investment cost, represent a somewhat restricted product segment. On the other hand, more complex technology often requires higher investment cost, but allows for wider end markets and possibly higher market price for some products. The available volume, its quality and nutritional properties, together with current legislation, regulate the potential use and processing demands for residual raw materials. In addition, market demand,

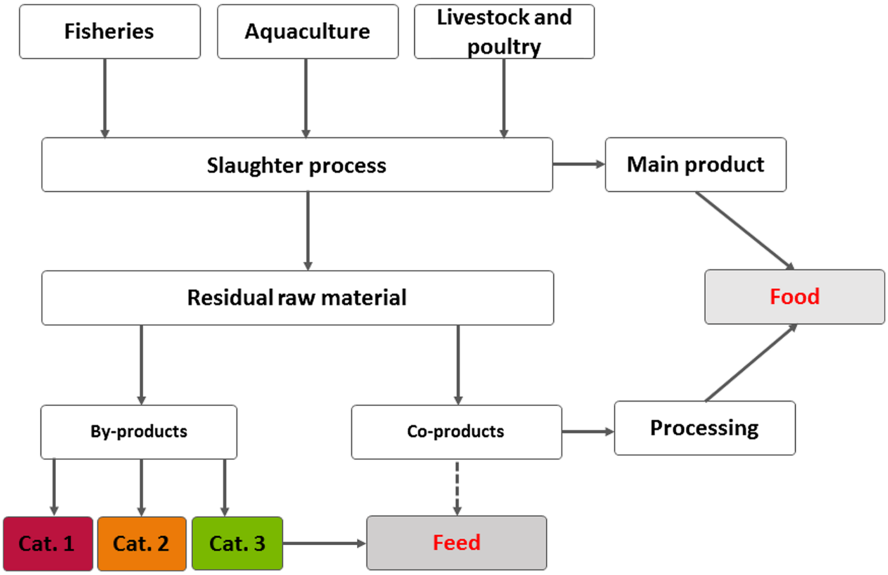

Fig. 1 Process from fisheries and farming of fish and livestock to co-products and by-products. Co-products are defined herein as residual raw materials from the slaughter process that still have food-grade quality and can be used for food. Other material not suitable for human consumption, for commercial or safety and/or regulatory reasons, is defined as by-products, separated into three categories (*1–3*) based on their origin and potential use in feed applications

consumer acceptance, feasibility, technology awareness, the required level of process control, and the available infrastructure influence the choice of the most beneficial and cost-effective process.

2 Volumes and Nutritional Values

The total global production of fish from aquaculture and fisheries in 2014 is estimated to be 128 million tons (Table 1) [2]. About 70 million tons of fish are processed as main products globally, indicating that residual raw materials constitute more than half of the entire fish weight. It is important to stress that, in some cases, discarded fish are not included in reporting of volumes of residual raw materials and hence represent large lost values, estimated at 7.3 million tons [3]. In Norway, white fish and pelagic fisheries resulted in 43 and 12 % residual raw materials in 2015, respectively [4]. Here, the most underutilized materials consist of heads, intestines and roe from whitefish, and blood from aquaculture processing [4]. Another example is tuna processing, where reported values range from 50 to 55 %, or up to 70 % [5].

Looking at meat, total production has reached more than 263 million tons annually [6]. Table 2 presents production of some major types of livestock together with the percentage of by-products resulting from processing of these species in Norway [7–9]. Other figures from the USA on the portion of residuals generated, given in live weight of livestock, are 49 % from cattle, 44 % from pigs, and 37 %

Table 1 Summary of production of freshwater, marine and diadromous (living in fresh and salt water) fishes in 2014

Continent	Inland waters		Marine areas				Aquaculture value (USD 000s)
	Sub-total all production (ton)	Sub-total aquaculture (ton)	Sub-total all production (ton)	Sub-total aquaculture (ton)	All production (ton)	Aquaculture (ton)	
Africa	4,527,980	1,682,039	5,455,976	12,814	9,983,955	1,694,852	3,573,168
Americas	1,607,879	1,076,073	13,870,982	1,018,460	15,478,861	2,094,533	11,112,732
Asia	47,557,457	40,319,666	38,759,848	3,388,124	86,317,305	43,707,790	73,760,477
Europe	831,604	477,051	14,205,697	1,820,109	15,037,301	2,297,160	12,099,014
Oceania	20,165	4,432	1,281,690	63,124	1,301,856	67,557	756,601
Summary					*128,127,276*	*49,861,891*	*101,301,993*

Data based on Food and Agriculture Organization of the United Nations (FAO) global production statistics [2], divided into continental and global data with a subset showing aquaculture production

Table 2 Production summary of major types of livestock by continent in 2014 based on Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) data [8]

Continent	Cattle 1000 head	Pigs 1000 head	Sheep 1000 head	Chickens 1000 head	Turkeys 1000 head
Africa	312,327	34,332	340,749	1,809,059	23,658
Americas	508,942	169,902	86,074	5,436,151	312,477
Asia	491,020	590,548	536,251	11,923,472	14,575
Europe	122,011	185,546	130,118	2,114,988	110,786
Oceania	40,226	5346	102,432	126,014	1377
Summary world	1,474,526	985,673	1,195,624	21,409 683	462,873
By-product (%)[a]	60	37	63	51	45

The final row shows the percentage of residual raw materials resulting from slaughter of each type of livestock based on Norwegian data [9]

[a] Figures based on the assumption that everything not sold as meat from the animals can be considered as by-product

from broilers [10]. Independent of the particular percentages, as seen for fish above, the amount of residual raw material is directly related to the physiology of each species; For example, pig slaughter results in low amounts of residuals because large parts of the skin and fat contain substantial amounts of easily solubilized collagen, used as a binding ingredient in sausages and other mixed products. For cattle, almost 20 % of the residual material consists of bones. Bone marrow contains large amounts of protein and was considered an excellent feed ingredient before the bovine spongiform encephalopathy (BSE) scandal in 1992. Nowadays, marrow bone is regulated as a specified risk material for destruction.

All these residual raw materials represent a different set of possibilities and challenges that it is important to investigate and solve before successful industrial processing. Although a large part of this biomass is utilized today in many countries, significant quantities are still under- or unutilized. Both animal and fish residuals have excellent nutritional value, and this material contains large amounts of high-quality protein, lipid, micronutrients and minerals that could be better utilized towards human consumption and other kinds of product. The protein content of meat-based residuals is generally 10–23 g per 100 g raw material, with porcine chitterlings and brains at the lower end and ears, feet and liver at the top end [11]. The protein in these different organs has different nutritional value and is more or less accessible, which is important from a processing perspective.

3 Regulatory Framework

The laws and regulations governing collection, transport, storage, handling, processing, use and disposal of residual raw materials are often-overlooked factors that severely influence the number of applications available for processing of this material. It is outside the scope of this review to cover all regulations worldwide; rather, we exemplify their importance by reference to European Union (EU) legislation. When material is found to be unfit for food but suitable for feed, it is classified as an animal by-product (ABP) and must be processed at appropriate by-product plants, according to EU regulations. After the material has reached such a facility, it can no longer be upgraded for food use. It is therefore imperative that as much as possible of residual raw materials is handled according to food hygiene regulations if the intended use is processing for human consumption.

The EU has developed an elaborate legislation framework governing the use of residual raw materials from fisheries, aquaculture, livestock, and poultry industries. The type and quality of each residual material are of utmost importance for its further processing possibilities and define its use for food or feed applications, according to the hygiene rules for food of animal origin [12]. Raw materials that do not meet the general rules for food hygiene or are classified as not suitable for human consumption are regulated by the rules of ABP regulations [13] and implementation of health rules for animal by-products and derived products not intended for human consumption [14]. ABPs can be divided into three categories (1–3; Fig. 2) based on their origin and potential risk to public and animal health and the environment. No ABPs can be used for human consumption, and only low-risk, category 3 by-products can be used for feed production. Some animal parts are not eligible for either food or feed use and are classified as category 1, specified risk materials. These include bone marrow, spinal cords and brains from cattle, sheep and goats, and sick and dead animals [13]. Category 3 by-products are further classified according to their origin, i.e. ruminants or non-ruminants, due to the risk of transmissible spongiform encephalopathy (TSE). A compilation of regulations for use of different category 3 ABPs in feed is presented in Table 3. ABP [13] together with TSE regulations [15] are major components of the EU’s strategy to eradicate feed-borne crises such as BSE in cattle, foot and mouth disease and dioxin contamination. The main objective of these regulations is to protect the population and farmed animals from any health risks related to contamination by infectious microorganisms or heat-stable bacteria-derived toxins (e.g. histamine and enterotoxins).

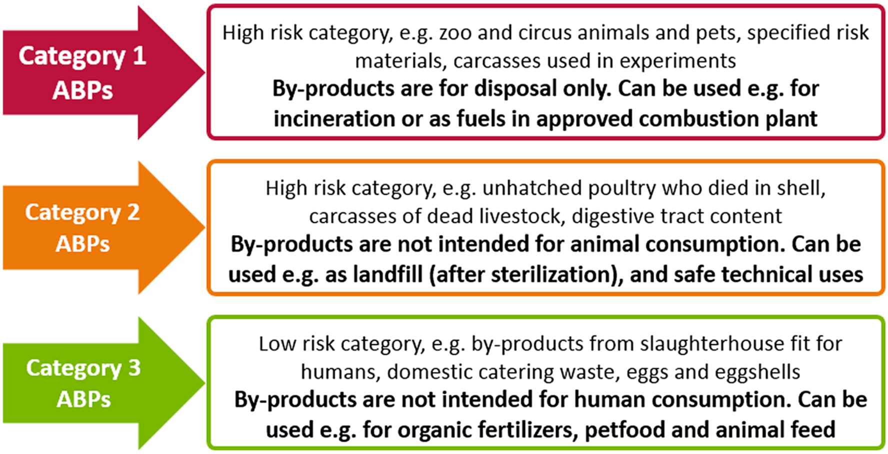

Fig. 2 Brief summary of by-products included in each of three animal by-product (ABP) categories regulated in the EU, and examples of some approved uses of ABPs in each category [13]

Table 3 Overview of category 3 animal by-product material from different animals suitable for feed use according to TSE regulation [15]

	Ruminants	Non-ruminants	Fish	Pets and fur animals
Processed protein from ruminants	✘	✘	✘	✔
Processed protein from non-ruminants	✘	✘	✔	✔
Blood from ruminants	✘	✘	✘	✔
Blood from non-ruminants	✘	✔	✔	✔
Hydrolyzed protein from ruminants	✘	✘	✘	✔
Hydrolyzed protein from non-ruminants	✔	✔	✔	✔
Collagen and gelatin from ruminants	✘	✘	✘	✔
Collagen and gelatin from non-ruminants	✔	✔	✔	✔
Fishmeal	✘	✔	✔	✔

Production plants that process category 3 by-products must comply with the general hygiene requirements provided in ABP regulations [13] and have a documented pest control program. Materials that have not received specific heat treatment during start-up or leakage must be either recirculated through the applied heat treatment step, collected and reprocessed, or discarded. The health rules regarding ABPs [14] describe several processing methods approved for heat treatment of category 3 by-products, based on methods 1–5 and 7 for material originating from domestic animals and methods 1–7 for aquatic animals (Table 4). The different heat treatment operation conditions are based on the following critical control parameters: (1) raw material particle size, (2) achieved core particle temperature level, (3) pressure, (4) duration of heat treatment and (5) in case of chemical treatment, the achieved pH level. In the case of fish processing, the material rapidly becomes tender as muscle proteins coagulate and disintegrate when exposed to mechanical forces in a cooker, strainer, screw-press or screw conveyor. Based on experience, reduction of particle size prior to the heat treatment step is not required to achieve uniform temperature throughout fish material [16]. Moreover, high shear forces during grinding might cause fat separation problems due to formation of emulsions and should be avoided if not needed.

4 Post-harvesting Handling, Industrial Processing and Analysis of Meat and Fish Residual Raw Materials

Both meat and fish residual raw materials represent a rich supply of easily available nutrients. However, they have high moisture content and are therefore easily spoilt in presence of microorganisms. Microorganisms can contaminate such materials via the fish and animals themselves, e.g. from the gastrointestinal tract or by contamination from hooves and/or hide and skin. Contamination can also come from the processing environment, e.g. due to poor employee hygiene or process facility cleansing routines. The commonest type of contamination is bacterial, but yeast and molds can also contaminate meat products. A broad battery of techniques are used post-harvesting for preservation of meat- and fish-based products [17], also

Table 4 Approved alternative methods for heat treatment of category 3 animal by-products [14]

Method	Particle size (mm)	Core temperature (°C)	Time (min)	Pressure (bar)	pH	Batch	Continuous
1	50	>133	20	3		×	×
2	150	>100	125	NS		×	
	150	>110	120	NS		×	
	150	>120	50	NS		×	
3	30	>100	95	NS		×	×
	30	>110	55	NS		×	×
	30	>120	13	NS		×	×
4[a]	30	>100	16	NS		×	×
	30	>110	13	NS		×	×
	30	>120	8	NS		×	×
	30	>130	3	NS		×	×
5[b]	20	>80	120	NS		×	×
	20	>100	60	NS		×	×
6[c]	50	>90	60	NS	<4.0	×	×
	30	>70	30	NS	<4.0	×	×
7[d]	NS	>76	20	NS		×	×
	NS	>70	20	NS		×	×

NS not stated

[a] Carver–Greenfield process, i.e. heating in a vessel with added oil

[b] The by-products must be heat coagulated and mechanically pressed to remove water and fat before final heat treatment

[c] Animal by-products originating from aquatic animals or aquatic invertebrates only

[d] Method approved by Norwegian authorities (wild fish >70 °C, aquaculture fish >76 °C; Nygård [16]), or any method authorized by the competent authorities complying with the following microbiological standards: *Clostridium perfringens* absent in 1 g product after heat treatment, *Salmonella* absent in final product ($n = 5$; $c = 0$; $m = 0$; $M = 0$), *Enterobacteriaceae* ($n = 5$; $c = 2$; $m = 10$; $M = 300$ in 1 g)

being valid for by- and co-products [18]. In industrial processing, conservation can be done before processing, but it can also be coupled with processing methods, e.g. in the case of fish silage. Conservation can also be minimized or potentially excluded in cases where the slaughterhouse is close by or even connected to the processing facility for residual materials. This ensures valorization of absolutely fresh residual raw materials.

4.1 Chilling and Freezing

Chilling and freezing are preferred methods in the meat and poultry industry to preserve the quality of main products. Cooldown is performed after processing and removal of the main product. By- and co-products from both fish and meat can be handled in the same way to preserve their quality, and chilling/freezing is easily facilitated in established slaughterhouses. Plate freezing equipment is applied to block-freeze mixed residuals sold as wet feed to the fur animal industry.

Fish residuals present some additional problems associated with conservation. Psychrotrophic microorganisms, which can grow at temperature as low as −5 °C, are present in fish from cold waters, affecting the temperature needed for effective conservation. Furthermore, the amount of microorganisms present in the digestive system of many fish species is dependent on season. Some smaller fish species such as capelin, herring, anchovy and sprat have high hydrolytic enzyme activity caused by active feeding on zooplankton during some periods of the year. Such species are prone to autolytic degradation and enhanced bacterial spoilage due to tissue softening and belly burst, which can be prevented by chilling.

Chilling of fish and residual raw materials onboard the fishing vessel is challenging. Many chilling techniques involve addition of water or ice to obtain heat transfer. Modern large-scale fishing vessels are normally equipped with active chilling systems based on circulation of refrigerated sea water or in combination with refrigerated fresh water to reduce salt uptake by fish. Low salt uptake is important to comply with fishmeal specifications regarding salt and ash content. Disinfection of pipes and the chilling system by ozone injection [19] or other antimicrobial agents improves the quality of recirculating water before addition of captured fish. This industrial practice has significantly improved the quality of pelagic fish caught for production of fishmeal and oil for use in animal feed, especially when combined with the optional addition of acetic acid. Chilling by addition of crushed ice is also possible but difficult to apply in large-scale operations. A promising option is production of ice slurry by partial freezing of sea water [20]. This technology results in pumpable ice and a very high chilling rate due to the content of small ice crystals and high cooling capacity. Excess blood-water generated by the above chilling technologies is generally drained off and pumped at sea. A novel cooling approach, avoiding addition of water, is use of solid carbon dioxide [21]. The choice of cooling medium and technology depends on several factors including the scale of the operation, the need for chilling to ensure quality before further processing, investments and energy consumption.

4.2 Organic Acid Preservation and Fish Silage

Preservation of meat- and fish-based foods using low-molecular-weight acids has been known for centuries [22]. Lactic, acetic, propionic, citric and benzoic acid are all organic acids used as food preservatives as well as being food ingredients, adding to their value and usefulness. This conservation principle is based on dissociation of the organic acid after diffusion through the microbial cell wall. This reduces the cytoplasmic pH, and in combination with accumulation of acid anions, inhibits cellular functions [22, 23].

Lactic and acetic acid are used to prevent contamination of freshly slaughtered beef carcasses after removal of hides and before or after evisceration [17]. Production of fish silage using organic acids is a well-established method in Scandinavia for preserving proteins and obtaining value-added products from fish raw material that would otherwise not be utilized. Fish silage can be described as a liquid product that develops when all or parts of the fish are treated with acid [24]. Use of fish silage in fish and animal feed has been widely studied, and it is regarded as a well-suited protein source [25, 26]. For preparation of fish silage, the raw material must preferably be crushed or ground and the mixture stirred to ensure good contact between the raw material and added acid. At acidic pH <4.5, enzymes naturally present in fish viscera degrade and liquefy the fish tissue without risk of bacterial spoilage [26]. A schematic overview of the production of crude silage is shown in Fig. 3. Further downstream processing of crude silage is covered in Sect. 4.5.2.

The rate of autolysis is determined by the content and activity of digestive enzymes, pH and temperature. Most commonly, formic acid is used, producing a stable silage at pH of about 4.0–4.5. Antioxidants, such as ethoxyquin, are added to prevent oxidation of fish oil. Production of fish silage is a relative simple and low-cost technology, but requires strict process control to avoid growth of spoilage bacteria. Moreover, this approach can be adapted to operations of any scale, and the final products are stable and can be stored for long periods. In Norway, production of fish silage is the main technique used for preserving marine by-products [4]. The final product is not suitable for human consumption, and silage technology should preferably be based on fish and residuals found unfit for food production. Although there have been reports of ensiling of chicken intestines [27], we are not aware of examples of industrial implementation of this technology for preservation of ABPs.

4.3 Salting and Production of Fish Sauce

Fish sauce is used as a condiment in large areas of South-East Asia. Conservation during production is achieved by addition of sea salt to fish raw materials. The mixture is stored for several (6–12) months at ambient (normally tropical) temperatures, until a clear, amber water solution, rich in hydrolyzed protein and salt, can be recovered. Very few microorganisms can survive and grow at such high salt concentration, and after a couple of months of storage, only low numbers of harmless halophilic bacteria are present. In addition to hydrolyzed protein and free amino acids, this liquid also contains short-chain fatty acids and aldehydes, adding cheesy and meaty aromas to the dominant sharp, salty taste [28]. To obtain good product stability, the amount of salt added to the fresh raw material must be in the range of 1:3–1:2 by weight. Normally, industrial production is performed in square concrete tanks covering large flat areas close to fishing harbours. In principle, fish sauce can be produced from most kinds of fish raw materials. Lean raw materials are

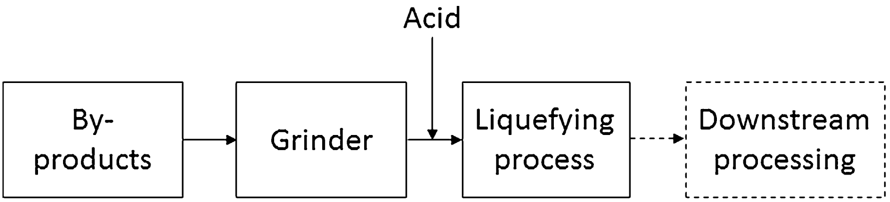

Fig. 3 Simplified illustration of silage process. The raw material must be ground for good contact with acid. At acidic pH, enzymes present in fish viscera degrade and liquefy fish tissue without risk of bacterial spoilage due to the low pH. The silage gradually liquefies due to the activity of tissue-degrading enzymes naturally present in the fish, mainly in viscera. Silage can either be used as is (crude) or be further processed (Fig. 5)

more suitable than fat raw materials, since the oil fraction does not contribute significantly to the volume of sauce recovered. Although fish sauce is normally produced from tropical fish species, pilot-scale experiments in Spain, Canada and Norway have shown that good-quality fish sauce can also be obtained from temperate- and cold-water fish species. Presence of intestinal tryptic enzymes, however, is of premium importance to achieve good protein hydrolysis and sauce recovery. There are considerable variations in the level of intestinal enzymes in small pelagic species, particularly in those from temperate and cold waters. To compensate for low levels of tryptic enzymes, suitable amounts of minced intestines from carnivorous white fishes can be added, since the intestines of such fishes always contain high levels of tryptic enzymes [29]. The major application of fish sauce is as a salting and flavouring condiment for vegetable dishes.

4.4 Rendering

Processing of animal and fish by-products is based on a common main principle called rendering technology. The raw material is heated to a temperature defined by legislation to eliminate any pathogenic bacteria, i.e. >70 °C for fish raw material and >100 °C for ABPs [14]. The main products from the rendering process are a protein powder and fat/oil. In this process, raw material is heat-coagulated followed by mechanical dewatering and separation steps to extract the oil phase, followed by thermal dewatering steps to concentrate the solubles and obtain dried high-protein powder [30, 31]. Such industrial-scale operations worldwide are fairly standardized, although some technology and process layout differences exist depending on the type of raw material and target product quality [32].

A general outline of the operation of such units for processing conventional fishmeal and oil is shown in Fig. 4. After heat treatment to 90–95 °C in a continuous screw cooker, the fish raw material is run over a strainer to remove free water and oil phase before entering a screw press. The compression ratio, normally 1:3.5–1:4 in a fish press, causes fish oil and water to be squeezed out of the coagulated material and through the sieve plates. The water and oil together with solubles and fine particles are collected in the bottom of the press and mixed with the oil/water phase removed by the strainer. The combined liquid streams are heated to above 90 °C and run over a decanter centrifuge to remove suspended fine solids before oil separation using a disc centrifuge.

4.5 Protein Hydrolysis

Protein hydrolysis is a method commonly used to extract proteins from meat and fish residuals. It involves breaking down proteins into smaller and more water-soluble peptides and free amino acids. The term “hydrolysis” literally means reaction with water, and protein hydrolysis requires presence of water molecules. The main purpose of hydrolysis is to increase protein recovery and the yield of valuable components. Protein hydrolysis can be achieved by chemical or enzymatic processes.

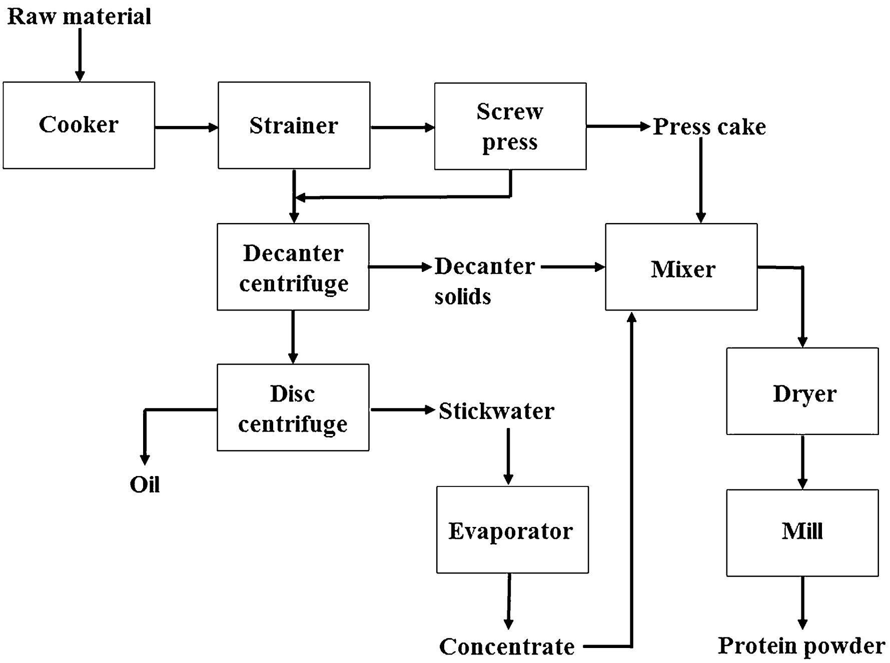

Fig. 4 Simplified process flow diagram showing the main unit operations applied in the wet rendering process. The raw material is cooked before entering the strainer. Decanter and disc centrifuges separate the solids, oil and stickwater phases. The stickwater is evaporated to a concentrate, mixed with the solids (press cake and decanter solids) and eventually dried to protein powder. The process conditions applied depend on the raw material and are listed in Table 4

4.5.1 Chemical Protein Hydrolysis

Chemical processing includes use of acid or alkali to cleave peptide bonds. Acidic protein hydrolysis is most commonly and frequently used to produce flavour enhancers from vegetables. Hydrolyzed vegetable protein is produced by treating the protein source with mineral acid (usually 4–6 M HCl) at 100–130 °C for 4–24 h followed by neutralization with NaOH [33, 34]. Alkaline hydrolysis is a straightforward process starting with protein solubilization by heat treatment followed by addition of alkaline agents (calcium, sodium or potassium hydroxide), adjustment of the temperature to a desired set point (usually 25–55 °C) and hydrolysis for several hours to achieve the desired hydrolysis product. Alkaline hydrolysis is less common in food and feed applications because of negative effects on the nutritive protein quality when using alkali. Thermal processing at alkaline pH can result in formation of toxic substances such as lysinoalanine, leads to racemization of L-amino acids to undesired D-amino acids and partly destroys the amino acids arginine, tyrosine, lysine, cysteine and threonine [35, 36].

Even though acid hydrolysis is preferred over alkali hydrolysis, the acid process can also influence the nutritive value of protein: the essential amino acids tryptophan and cysteine are destroyed, and glutamine and asparagine are converted to glutamic acid and aspartic acid [37]. Moreover, neither acid nor alkali hydrolysis is specific, and both generate large amounts of salt in the final product after the neutralization process. In general, processes based on chemical hydrolysis yield hydrolysates with reduced nutritional quality and poor functionality that are restricted to use as flavour enhancers [36]. Chemical hydrolysis involves use of highly corrosive acid or base and requires glass-lined stainless-steel reactors that can withstand high pressure and temperature [37].

4.5.2 Enzymatic Protein Hydrolysis

Production of protein hydrolysates using protein-digesting enzymes, i.e. proteases, is a very promising alternative for valorization of proteins from meat and fish by- and co-products for different markets. The process used in this technology is regarded as mild and results in high product yield without prejudicing nutritional quality by e.g. destroying amino acids as seen with chemical hydrolysis. Enzymatic hydrolysis decreases the molecular weight of intrinsic proteins and peptides and increases the number of ionizable groups, resulting in new peptides that are smaller and more water-soluble than the intact proteins [38]. A typical hydrolysis process is characterized by an initial rapid burst phase where the substrate is in excess. As the enzymatic hydrolysis reaction progresses, the reaction rate levels off. This can be explained by reduced enzyme activity caused by one or a combination of the following factors: change in reaction pH, fewer peptide bonds available for cleavage [39], substrate [40] and product inhibition [41], as well as the possible presence of protease inhibitors in the substrate [42].

Enzymatic hydrolysis can be performed either by use of (endogenous) enzymes that occur naturally in the substrate or by addition of commercially available (exogenous) enzymes. Production of fish silage is essentially endogenous enzyme protein hydrolysis using visceral digestive enzymes. As described in Sect. 4.2, the organic acid preserves the fish by-products, but lowering the pH also serves to activate digestive proteases from the fish. Use of endogenous enzymes is seen as an inexpensive and mild process; however, it usually requires long hydrolysis times and results in nonspecific hydrolysis. Hence, use of exogenous enzymes is considered the best choice for producing food-grade protein hydrolysates, as the process is highly specific and reproducible and may enable tailoring of well-defined hydrolysate products. Generally, the additional cost associated with use of the latter type of enzymes is considered justifiable in the enzymatic hydrolysis industry. This is because the specificity and higher reproducibility enable production of products with potential to reach higher-paying markets, compared with products based on e.g. rendering or ensiling. There are a vast array of commercially available proteases, and a judicious choice should be made with regards to enzyme performance and cost [43].

Proteins are complex substrates containing peptide bonds with different accessibility to enzymatic cleavage [44]. Proteases are ubiquitous and exhibit a huge diversity of action, e.g. in terms of substrate selectivity, where individual proteases cleave the peptide bond, and regarding pH and temperature preference [45]. In processing of protein-rich materials, protease selection is based on both product parameters, such as peptide size distribution, and the protein source [37]. Processing parameters also determine product outcome, e.g. enzyme-to-substrate ratio, pH and processing time. Careful optimization of these parameters enables development of products with selected properties.

A simplified protein hydrolysis process flow diagram is shown in Fig. 5. The raw material is minced and diluted with water to ensure good mixing and enzyme access [46]. Proper dilution can prevent product inhibition and maximize product yield, but added water is also a factor influencing processing costs associated with the drying process. A compromise between the desired product yield and the amount of water to be removed is imperative. The pH of the enzymatic hydrolysis process is often selected based on the optimum for a given protease [47]. However, adjusting the pH requires acid or base, which can result in high levels of salt in the final hydrolysate. This may reduce the nutritional value of the product and, if possible, should be avoided. At the end of the reaction, enzyme activity is terminated by its irreversible denaturation by heating to above 90 °C for at least 10 min. The crude hydrolysate is separated by a three-phase decanter into an oil, water and solid phase. The water phase constitutes water-soluble protein hydrolysate that can be concentrated in an evaporator followed by drying in a spray dryer. The solid phase contains bones and insoluble proteins that can be used in production of bone meal.

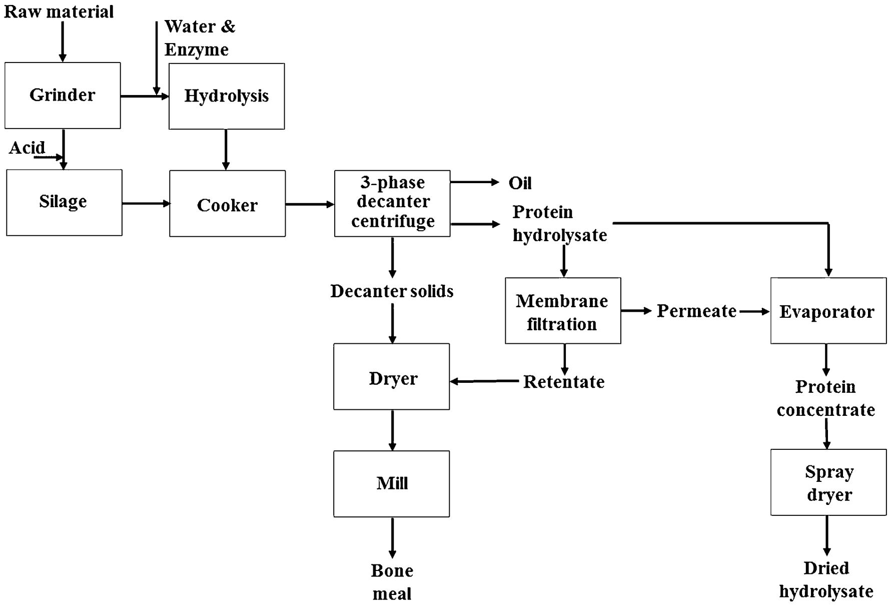

Fig. 5 Simplified process flow diagram showing the main unit operations applied in a hydrolysis process. The raw material is ground and mixed with water and enzyme, and the hydrolysis process is run using predefined time and temperature conditions. In case of silage production, acid is added to the raw material followed by hydrolyzation using inherent proteolytic enzyme activity at ambient temperature. Enzyme activity is terminated by cooking, and the crude hydrolysate is separated by three-phase decanter centrifuge into oil, soluble peptides and amino acids, and solid phases. The solids are dried and milled into bone meal. The protein hydrolysate can be membrane-filtrated to achieve desired a molecular weight distribution of peptides and eventually evaporated and dried to obtain dry protein hydrolysate

4.6 Analysis

Valorization of proteins from by- and co-products is, in most cases, based on heterogeneous raw materials with substantial and uncontrollable variation in quality. It is crucial to develop a production process that is robust towards raw material quality and variations in order to produce a stable product. An essential first step is thus evaluation of the raw material composition (e.g. fat, protein, moisture and ash). The Kjeldahl nitrogen method for protein determination is a commonly used analytical approach based on classical wet chemistry. The method provides figures for the total nitrogen content in a sample, providing the protein content after multiplication by a suitable nitrogen-to-protein conversion factor. Food matrices contain other nitrogenous organic compounds that will influence the determination of the true conversion factor, such as non-protein amino acids and nucleotides. Substrate-specific nitrogen-to-protein conversion factors have been calculated for poultry by-products, meat and bone meal, and Atlantic salmon head and backbone residuals [43, 48]. Another important parameter for characterization of raw material is the amino acid composition. Amino acid analysis is typically performed using chromatographic methods and can provide important knowledge about the nutritional properties of the raw material.

The above analytical measurements are performed offline and typically on a small representative sample from a batch of raw material, and the heterogeneity of meat- and fish-based products poses a significant challenge for representative sampling. Rapid and non-destructive spectroscopic techniques such as fluorescence, Raman and near-infrared spectroscopy have been demonstrated to be valuable tools for characterization of raw materials and products in terms of gross composition [49, 50]. Such approaches are expected to be essential for enzymatic protein hydrolysis in the future, where novel strategies for optimization and monitoring are used to obtain robust processes and products with defined quality.

One of the main control parameters in protein processing is the measured extent to which protein has been degraded. In this respect, measurement of the degree of hydrolysis, i.e. the percentage of cleaved peptide bonds, is a widely used approach for monitoring enzymatic protein hydrolysis. Another valuable parameter used for characterization of protein hydrolysates is the molecular weight distribution of the peptides. Unlike the degree of hydrolysis, which is always relative to the starting material, the molecular weight distribution is a direct measure of the peptides and proteins present. A major limitation of both of these measurement techniques is their laborious sample treatment and lengthy analysis time, limiting their use in industrial settings and as potential online monitoring tools. Recently, it was shown that Fourier-transform infrared (FTIR) spectra are useful for characterization of enzymatic protein hydrolysates [51, 52]. This technique has promising potential as an on- or at-line process monitoring tool for measurement of degree of hydrolysis. In the future, FTIR could thus be a valuable tool in industrial hydrolysate production by providing online process control, optimization possibilities and thus stable product quality.

Thorough characterization of individual peptides is also a very important aspect, especially if the chemistry of a given product is to be related to a specific sensory or biological activity. In such cases, chromatographic fractionation and mass spectrometry has been shown to be a powerful technique for unequivocal elucidation of peptides [53].

4.7 Scale-Up of Bioprocesses

Many of the processes used to convert fish and animal residuals to higher-value products are developed at laboratory scale and demonstrated at pilot scale. When developing new products from these biomasses, many processes fail to meet significant challenges in the transition from laboratory to industry scale. Thus, the upscaling steps need to be carefully planned already at the start of product development. The scale-up phase is commonly called the demonstration phase, where a prototype product can be produced, capital and operating costs of the process calculated and the product tested in the market. This is an important step in bioprocess development. One of the reasons for these challenges is the chemistry during process scale-up, where differences in mixing, shear rate, and mass and heat transfer result in differences in how the biomass is processed compared with small-scale processes. In addition, production of process inhibitors that are effective at large but not small scale can be experienced [54, 55]. Another challenge is the economics of the demonstration phase, which is referred to as the "valley of death" in product development. There is a lack of available risk capital to perform this step, and limited access to demonstration plants that can be used to test processes. Demonstration plants are needed to reduce the risk and cost of the demonstration phase. It is too risky for most developers to invest in a full-scale facility before the process or market acceptance has been demonstrated. A solution that has been successful in many countries is the establishment of publicly financed demonstration plants. Here, flexible plants are built and constructed to accommodate a large variety of different processes. In such plants, many different developers can test and demonstrate their process and the product in the market. Due to the flexibility of such plants, operating and capital costs can be estimated; however, loss of biomass and process yield will likely occur when using such flexible plants, which can be improved in a plant specially designed for one process. Still, publicly financed demonstration plants have been and will be important for continued commercialization of new biomasses towards new products [56].

5 Applications

Co- and by-products from fish and animal processing have great potential for use in food and feed products, as well as for other markets. For human consumption, it is important that residuals are not classified as ABPs. As described in Sect. 2, in general, co- and by-product materials contain high amounts of protein, with essential amino acids, vitamins and minerals.

5.1 Food Ingredients

Fish and meat co-products have several applications in food ingredients, if the process implements systems such as good manufacturing practice (GMP) and hazard analysis and critical control point (HACCP) [57]. Variety meats and other parts of animals that are traditionally considered edible, such as kidneys, liver and oxtail, can be used directly for human consumption. Collagen-rich material such as animal hides and bones and fish skin are used for gelatin production. In Norway, dried cod heads, co-products from the stock- and klippfish industry, and meat-rich salmon trimmings and backbones are sold for different food applications. There are also many different applications of blood in food production, e.g. as an emulsifier, stabilizer or clarifier [58, 59]. However, to use animal blood for human consumption, it must be extracted using special equipment in direct contact with a cooled tank to avoid contamination [60].

For some time now, production of enzymatic protein hydrolysates has attracted interest for use in human nutrition. The process is mild and does not impair the nutritional quality of the original protein substrate. Protein hydrolysates may have several applications as food ingredients, e.g. as emulsifiers or foaming agents. Other applications are in specialized adult nutritional formulas, e.g. in diets for the elderly who need extra protein supplements to maintain their body weight, formulas for infants with allergies to intact food proteins or with congenital metabolic disorders, and nutraceuticals [61–64]. A current drawback with production of protein hydrolysates is the generation of bitter and unpalatable tastes during the hydrolysis process. Bitter taste is mainly ascribed to small peptides of less than 1000 Da with hydrophobic and/or aromatic amino acids [65]. Not only the presence of hydrophobic and aromatic amino acids, but also the amino acid peptide sequence is important for the intensity of such bitter taste. Based on the hydrolytic specificity of the protease chosen, it may be possible to produce hydrolysates with different bitter potency from the same substrate [66]. It may also be possible to remove the bitter taste using different debittering techniques [67], although such techniques may be challenging in industrially relevant applications [68]. In general, restricting the hydrolysis to reach a low degree of hydrolysis with a broad molecular weight distribution will reduce the formation of bitter taste [66]. In addition to formation of bitter taste, protein hydrolysates have flavours related to the raw material, i.e. fish, chicken, meat etc., which influence the overall flavour profile. These flavours are not related to the protein, but rather water-soluble compounds present in the substrate [66].

5.2 Feed

Use of processed animal proteins in fish feed has several advantages compared with currently used plant proteins, as plant-derived feed ingredients may contain anti-nutrients and allergenic proteins [69]. Meat and bone meal and fishmeal are the final products from rendering of animal and fish by-products, respectively (Sect. 4.4). These are excellent feed sources due to their high content of essential proteins, minerals and vitamins. However, especially meat and bone meal may be subjected to large variations in nutritional quality. This is mainly caused by variations in the composition of the raw material and the harsh rendering temperatures applied [70, 71]. In addition, raw materials rich in bone will result in meal with high ash content, which is associated with low protein digestibility.

As described in Sect. 4.5.2, enzymatic protein hydrolysis results in two fractions with usable peptide content. One is a water phase with soluble peptides, and the other is a solid phase containing insoluble proteins and minerals. Both are frequently used as feed, although with large price differences. Protein hydrolysates have favourable formulation properties for animal feed, such as high solubility over wide ranges of pH and ionic strength, and a set of positive nutritional properties, including feeding stimulation and palatability enhancement, facilitated adsorption of e.g. labile and insoluble amino acids, and presence of beneficial hormone-like peptides [72]. As mentioned above, feed production is an area subject to a strict regulatory framework; a relevant review of the European regulatory framework and potential uses of ABPs for feed was published recently [73].

The potential valorization of proteins from fish residuals is not fully exploited [74]. In general, fish protein products have many applications within feed for the aquaculture sector and monogastric land animals such as weanling pigs, poultry and pets. The use and importance of fishmeal in the aquaculture sector have grown substantially over the last decades [75], and there is huge potential for increased fishmeal production from underutilized sources for this sector. Meat and bone meal from pigs and poultry may also have greater potential as feed ingredients within the aquaculture sector. In Europe, use of ABPs in fish feed has been restricted due to the risk of TSE, but these restrictions were lifted in 2012 for non-ruminant protein meal [76]. Recent studies evaluated the effects of poultry and porcine by-products as feed ingredients for Atlantic salmon and found that ABP material can provide about 50 % of dietary protein without negative effects on growth. Moreover, use of ABP protein did not show any severe negative effects on gut health, which is often a problem with plant-based diets [77, 78].

Piglets show greater preference for feed that includes either dried hydrolyzed porcine protein or fishmeal compared with other protein-rich feeds, e.g. soybean protein, wheat gluten and sweet milk whey. Feed based on fishmeal got the highest score at inclusion level of 50 g per kg, with high preference for feeds including hydrolyzed porcine protein over a wide inclusion range (50–200 g per kg) [79].

5.3 Pet Food

Companion animals, such as cats and dogs, represent additional consumption of protein via human purchasing. As such, this also represents an additional indirect protein need for humans. To ensure sustainable pet ownership in the future, pet food must be sustainable and affordable and effectively satisfy the requirements for good animal health and well-being [80]. In general, all category 3 by-product material is suitable for pet food production (Table 3). In production of wet pet food, only residuals that are eligible for human consumption (i.e. co-products), but found unfit for various reasons, can be used [13]. Pet food formulas are either dry, semi-dry or wet, and protein content varies between 10 and 50 %, with wet foods at the lower and dry food at the upper end [81].

Meat and bone meal products derived from rendered ABPs are used by many pet food producers. However, their popularity is declining due to several reasons, including the name of the product and its perceived association with TSE risk. Also, poultry meal is a widely used protein ingredient in pet foods. In general, poultry protein meals are well utilized by dogs and cats and make up a large share of the total protein in many premium pet foods [82]. Protein hydrolysates of both animal and fish origin are also increasingly utilized for pet food applications. These hydrolysates contain short peptides and free amino acids that might act as feeding stimulants and palatability enhancers [71, 83]. Moreover, protein hydrolysates might have hypoallergenic [84] and bioactive properties (as discussed below), making them interesting pet food ingredients for companion animals with special needs.

5.4 Health-Promoting Products

By- and co-products from the fish and meat industry are rich sources of biologically active molecules with potential health-promoting effects. Such bioactive molecules can be included in e.g. food (nutraceuticals) and as active ingredients in cosmetics (cosmeceuticals). Bioactive peptides (BAPs) are short chains of amino acids with hormone- or drug-like activity that modulate physiological functions through interactions with specific therapeutic targets [85]. Numerous BAPs derived from co-products have been proven to exhibit a wide range of positive health effects, with most such research focussing on blood pressure lowering, blood sugar regulation, and anti-microbial and anti-oxidant activities [86–88]. In addition to a specific therapeutic function, peptides may have other beneficial effects; for example, rats fed with hydrolyzed fish protein showed reduced visceral adipose tissue mass [89], and peptides derived from collagen were shown to increase muscle mass and strength in elderly men [90]. BAPs from by-products can also have a positive effect on collagen production, which makes them attractive ingredients in cosmeceuticals for wound healing and skin aging [91].

A standard process for evaluating the health-promoting potential of BAPs comprises several stages of analysis involving both in vitro and in vivo experiments. Ideally, to validate the bioactivity of a given bioactive peptide, human intervention studies are necessary. However, such studies are expensive and typically conducted after selecting a potent candidate through a rigorous screening process. The majority of screening experiments for BAPs are performed using in vitro assays including enzymatic assays, cell cultures, genomic tests and in vitro digestion stimulation. Candidates identified through such screening exercises are further evaluated using animal models and eventually human intervention studies (Fig. 6).

A large proportion of the reported bioactivities of meat and fish protein hydrolysates are based on either chemical or enzyme-based bioassays. BAPs in food must be absorbed in the intestine during digestion, and enter the bloodstream to exert their physiological effect, although some peptides may act locally in the

Fig. 6 Comprehensive method platform for testing health-promoting ingredients, from preliminary in vitro digestion models, bioactivity screening assays, to extensive human intervention studies

stomach or intestine. After absorption, BAPs can act on a given therapeutic target as a single molecule or synergistically [92]. BAPs from different meat and fish processing co-products have been shown to possess bioactivities towards key therapeutic targets related to diseases such as diabetes, obesity and coronary heart disease [53, 93–95]. Cell models can provide additional knowledge after preliminary bioactivity screening assays, including endogenous effects, dose-response and target organ. Some peptides exert their effect on muscle development, while others influence liver, bone, angiogenesis, inflammation etc. It is therefore vital to use different types of cell model when examining such bioactivity effects.

5.5 Other Applications

It is important to stress that there are a huge number of applications for products based on meat and fish residual raw materials that are not covered above. Protein hydrolysates based on both chemical and enzyme production can be used as growth media in areas of fermentation and biotechnology for production of pharmaceuticals and recombinant proteins, as well as in diagnostic media [96]. Blood from various animals has also shown antioxidant and antimicrobial activity [97]. Also, bovine serum albumin extracted from blood is an important tool in microbiology and many enzyme assays. Various enzymes extracted from livestock livers are used in many biotechnology applications, e.g. several types of dehydrogenases and catalase from bovine liver and porcine liver esterase. Aside from being an excellent feed source, poultry feathers can be used for a range of non-food applications [98]. Examples span electrical and electronic applications, composite materials, oil adsorbents and generation of micro- and nanoparticles. Meat and bone meal and fat from the rendering process may also be used as fertilizers and biodiesel or as raw materials for the chemical industry, respectively [99].

6 Utilization of Co-products towards Consumer Products

In valorization of meat and fish by- and co-products, the human consumption market has been specifically challenging to reach. In the meantime, consumers are becoming increasingly concerned about the environment and sustainability. This trend is expected to continue or even increase in the future, with consumers aiming to influence industries via their consumption pattern, i.e. food, and particularly protein production [100]. Being an integral part of the value chain, consumers influence both the levels of food waste and the acceptance of value-added co-products [101]. Still, challenges remain regarding regulations and the feasibility of introducing products for human consumption in terms of supply, control and economics [56]. Only minor efforts have been made so far to understand the best approaches for upgrading fish- and animal-based co-products to lucrative products for human consumption [102].

Fish- and animal-based by- and co-products can be used in development of new products or to replace ingredients in existing products. Much of this work is following an “industry push” strategy, where products are tested with consumers after most product development decisions have already been made [103]. Currently, there is a lack of awareness regarding the need for consumer acceptance, which is of utmost importance for market success of potential products based on ingredients from new sources [104, 105]. Thus, poor product development strategies can lead to product failure. To improve this strategy, implementation of systematic product testing will likely increase the chance of market success [106].

Information about products and production methods have repeatedly been shown to influence consumer choices [107]. Studies on use of added-value compounds usually rely on analytical and sensory testing processes that do not take into account the consumer perspective [108]. In rare cases, consumer acceptability studies are included, but usually on end products and without providing information about the origin of the ingredients [109]. When excluding information about origin in use of trained panels or consumers for tasting of food products, it is hard to estimate the actual acceptability of a product by consumers in the real market. Provision of relevant information as part of a balanced communication strategy could increase consumer demand for products with proteins from production methods with reduced environmental impact [110].

Consumers have adequate knowledge on how to use food ingredients and supplements, but besides segments of particular interest, they are rarely aware of product production methods [111, 112]. When new products or ingredients are presented to the market, consumers become more alert and curious regarding their origin [113]. This concern has been one of the main barriers to e.g. use of ABPs or genetically modified ingredients in salmon feed, despite the potential benefits [114]. Considering the fact that consumer acceptance varies and changes with exposure to products and information, it is possible to achieve potential improvement of products without sacrificing quality [115].

When consumers analyze their food choices, they are confronted with trade-offs between convenience, health and sustainability that may challenge their final decisions [116]. Thus, communicating one positive element of a product, such as “sustainability” or “using the whole animal”, should not come in the way of aspects such as “food safety”, “health”, “convenience”, “hedonic expectations” etc. In fact, it is more probable that products will be chosen by consumers if all positive elements of the product are combined with production information in a holistic and transparent reputation-building strategy. Such a strategy could be successful when used in combination with targeting of consumers that are interested in functional foods and likely to seek additional information about the product [117].

Despite continuous improvements, there has been an increase in public concerns about livestock and aquaculture production, due to food crises and the environmental impact of production practices [118]. This has led consumers away from consumption of meat proteins in favour of plant proteins [119]. An expected positive trend in the near future is that consumers will demand fish that is farmed under safe and controlled conditions in clean waters [120]. Therefore, any information supplied to consumers should focus on trust building, with transparent and balanced communication. This type of approach could establish a fertile ground for introduction of new products and ingredients in the market for human consumption, such as products based on sustainable co-products from fish and animals.

7 Challenges and Future Trends

There are a growing number of initiatives in the context of the foreseen transition from an oil-based economy to a bioeconomy. This includes concomitant awareness of consumers, producers and governments about the importance of recovery and recycling of what was previously regarded as waste. In many countries worldwide, research funding is being directed towards finding new methods to optimize recovery and exploitation of intrinsic raw material components, preferably using a biorefinery approach to enable maximum utilization. There is immense potential for growth when it comes to increased valorization of fish- and animal-based co- and by-products. As more products reach the market, producers will start competing for what are now inexpensive starting materials, and one can expect higher prices for residual materials in the future. At the same time, producers must be able to rely on a stable supply of raw materials to obtain the predictability required for production.

Many of the technologies described above form the basis of established industries, with well-established processing operations and well-known markets. Enzymatic protein hydrolysis represents an up-and-coming, relatively new industry based on a highly enabling technology, showing great promise based on published research. However, when implemented in industrial practice, results show that there are challenges related to controlling the number of variables that affect the properties and quality of the final product. This includes raw material variations, processing parameters such as pH, time, choice of enzyme and inactivation method in protein hydrolysis, choice of downstream unit operations and choice of drying method. Current industrial practices rely on traditional and established analytical tools for quality parameter evaluation. However, this methodology cannot provide the fast feedback required for production of products with specific properties. In the future, nondestructive spectroscopy-based technologies could be valuable tools for industrial production of protein hydrolysates, by providing online process control, new optimization possibilities and reduced product quality variation.

Considering the predictions of future food shortages, it makes sense to strive to use as much of high-quality meat and fish co-products for human consumption as possible. There is increasing interest in upgrading such residual materials for human consumption; however, consumer acceptance and preferences should be known and targeted before products reach the market. Sometimes the desired market growth is hindered by a lack of synergy among stakeholders, and a lack of common vision. Achieving perfect collaboration among all stakeholders is challenging, but increased cooperation will lead to shared vision, strategic planning, targeted communication and overall image improvement.

Image improvement forms the foundation for societal acceptance, and vice versa, societal perceptions can also inform strategic decisions of stakeholders towards image improvement and positive reputation. Based on systematic research, stakeholders will reach a point where there is less "waste products" and "residual raw materials" but rather optimized utilization and processing of all resources.

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Compliance with Ethical Standards

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